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EXPERIMENTAL ULCERS INDUCED BY FORCED IMMOBILIZATION IN THE WHITE RAT

II. ANATAMOPATHOLOGY OF THE GASTRIC LESIONS DEVELOPMENT OF ULCERATIONS AFTER ENDING IMMOBILIZATION

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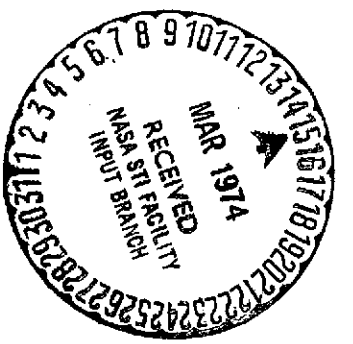

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16. Abstract A study is made of certain aspects of gastric ulcers induced by forced immobilization. The macroscopic appearance was found to be non-specific. The lesions were found to be multiple in 67-90% of the cases; there was no correlation between precise procedure and the incidence or multiplicity of lesions. The healing process of the lesions was studied by sacrificing animals at intervals after terminating the immobilization. Complete healing had occurred by the ninth day in 80% of the animals, and there was no chronic ulceration.

Microscopic study showed the fundamental lesion to be a slough which detaches, leaving the ulcer. Predominant in the histologic pattern were vascular changes in the form of "capillary pits." Glandular redifferentiation occurred next to the formative scar.

Associated visceral lesions included fatty infiltration of the liver as well as the presence of vacuoles in the renal tubular epithelial cells.

The results are discussed with particular attention to the use of this method as a pharmacodynamic test.

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EXPERIMENTAL ULCERS INDUCED BY FORCED IMMOBILIZATION IN THE WHITE RAT

II. ANATAMOPATHOLOGY OF THE GASTRIC LESIONS

Detailed anatamopathologic study is indispensable as a means /888* of analyzing experimental ulcers. Undoubtedly the first aim of this type of study should be to determine the number of ulcers, their extent and the exact depth of tissue destruction; in this area microscopy will frequently correct first impressions obtained by mere macroscopic observation.

Such study also provides a means of determining the actual mechanism of the gastric lesion by combining various types of observation: (1) of the ulceration itself, noting the presence or absence of inflammation; (2) of the vicinity of the ulceration: the extent of vascular reaction, the pepsinogen content, the comparative alteration of various types of cells; (3) by sacrificing animals at intervals it is possible to find the origins of changes occurring prior to the ulceration and to differentiate, for example, lesions primarily caused by a chlorhydropeptic attack in the cells, initially linked to a parietal vascular disturbance; (4) the pathologic anatomy of organs other than the stomach may indicate the presence of a general disturbance, revealing the gastric lesion as a mere epiphenomenon in a larger reactional mechanism.

These were the objectives of the present study, with reference to experimental ulcers induced by forced immobilization in the white rat [3]. Moreover, since the technique used gave a high percentage of lesions with relatively low trauma, it was possible to study the manner in which the gastric lesions healed and the time

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*Numbers in the margin indicate pagination in the foreign text.

required for healing by sacrificing series of animals at varying intervals after ending the immobilization.

Technique

The technique for inducing ulcers by forced immobilization was discussed in detail in a preceding article [3].

Results

Consideration will be given to: (a) the sum total of macroscopic observations, that is, observations of some lesions at the end of the immobilization period and others during the healing process; (b) microscopic analysis of these changes; (c) extragastric visceral lesions.

A. Macroscopic Study of the Gastric Lesions

1. Lesions at the end of immobilization (7 or 24 hours). If the stomach was ulcerated the lesions were identical in appearance whether the immobilization period had been 7 or 24 hours.

(a) Appearance and site. The ulcerations were always located in the glandular -- or ventricular -- part of the stomach, which they were able to reach at any point on its surface.

They were generally quite deep, hematic, with a black fundus; the edges were polycyclic or in uneven "shreds," not raised or congestive. No perforations were observed.

Less often there was only a hematic but still interstitial infiltrate of black blood, covered by a more or less thick layer of mucus; discrete pressure on this magma resulted in its elimination and separated the ulceration.

The ulcers were almost always multiple and unequal in size. /589 the principal lesions were most commonly located on the anterior

and posterior surfaces in the form of striae 5 to 6 mm in length and 1 to 4 mm in width, following the axis of the curvatures.

In the rest of the ventricle the lesions were usually smaller, punctiform, and at the limit of visibility, about the size of a large pinhead.

Aside from the ulcerations, other modifications of the mucosa could be observed: desquamative gastritis, purpura and especially punctiform hemorrhages of the type which have been described as "capillary pits."

It should be noted that these visible characteristics are definitely not specific for ulcers induced by immobilization, but are, rather, quite similar to those observed in response to phenylbutazone [2], cortisone and delta-cortisone [1]. The blackish coloration and polycyclic shape of the ulcerations, their location and their tendency to have symmetrical surfaces are also found in ulcers produced by pyloric ligation (Shay's ulcer [5]), but the site of this type of lesion is the rumen.

(b) Number. When study of experimental ulcers is being applied to therapeutic research, some investigators prefer to evaluate their results by a count of the number of lesions or surface area covered.

The lesions observed in the present study were therefore analyzed from this standpoint.

Percentage of animals with only a single gastric ulcer:

Immobilization for 24 hr.....22%
" " 7 hr.....19.8%

Immobilization for 7 hr, nephrectomy.....8.3%
" " 7 hr, 10 mg cortisone....33%

Average number of ulcerations in cases of multiple gastric lesions:

Immobilization for 24 hr.....5.2%
" " 7 hr.....2.5%
" " 7 hr, nephrectomy.....3.2%
" " 7 hr, 10 mg cortisone....2.8%

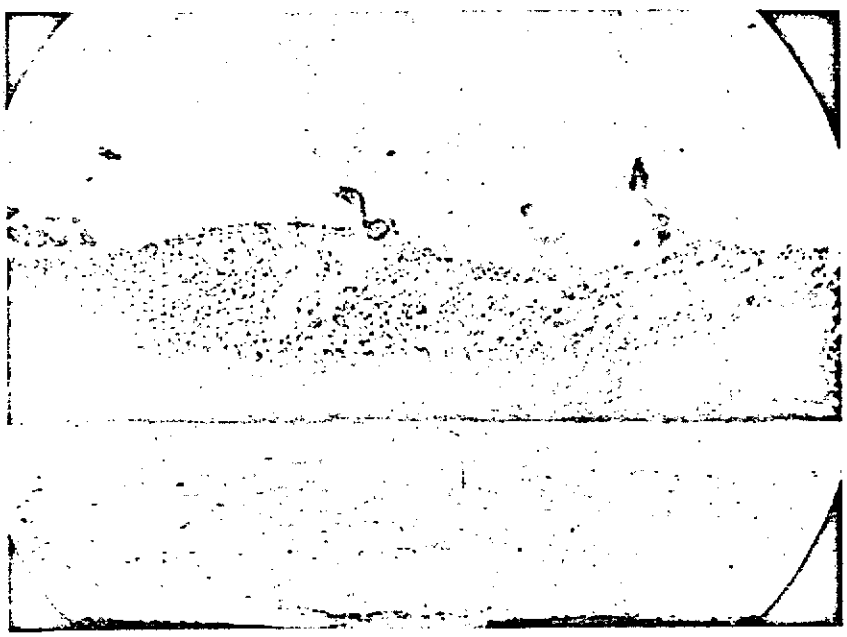

Fig. 1. Ulcer after 24 hours of forced immobilization. Slough in course of detachment.

A comparison of the above figures with our earlier results [1] shows that there is no correlation between the overall incidence of ulceration for a given experimental procedure and the number of ulcers per animal in the same series. /890

For example, of the rats on whom nephrectomies were performed after seven hours, 36% had ulcers but only 8.3% had a single gas-

Fig. 2. Slough, initial stage.

tric lesion, while control animals examined after 24 hours showed lesions in 86% of the cases, but single lesions in only 22%.

A count of multiple lesions showed that the control animals immobilized for 24 hours had both a much higher percentage of lesions (86%) and a much greater number of gastric lesions per animal [4]. On the other hand this correlation definitely did not hold true for the aggregate of series in which a seven-hour immobilization period was used.

The most significant phenomenon would thus appear to be the presence or absence of ulcerations, with their number dependent only on the immobilization period and not on the nature of the experimental procedure.

2. Development of lesions after end of immobilization. (a) Study technique. After 24 hours of immobilization each animal was released and placed in a cage with other rats in the same series having undergone the same process. They were given food and water, as were the control animals. After three or four hours they resumed normal motor activity.

These animals were sacrificed between the first and the ninth days, depending on the time at which they were released.

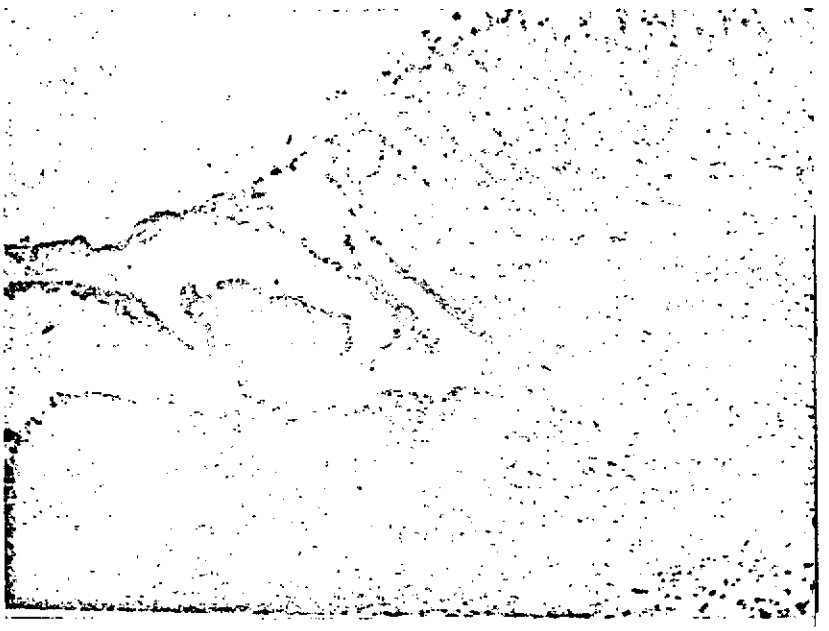

Fig. 3. Healing ulcer (third day).

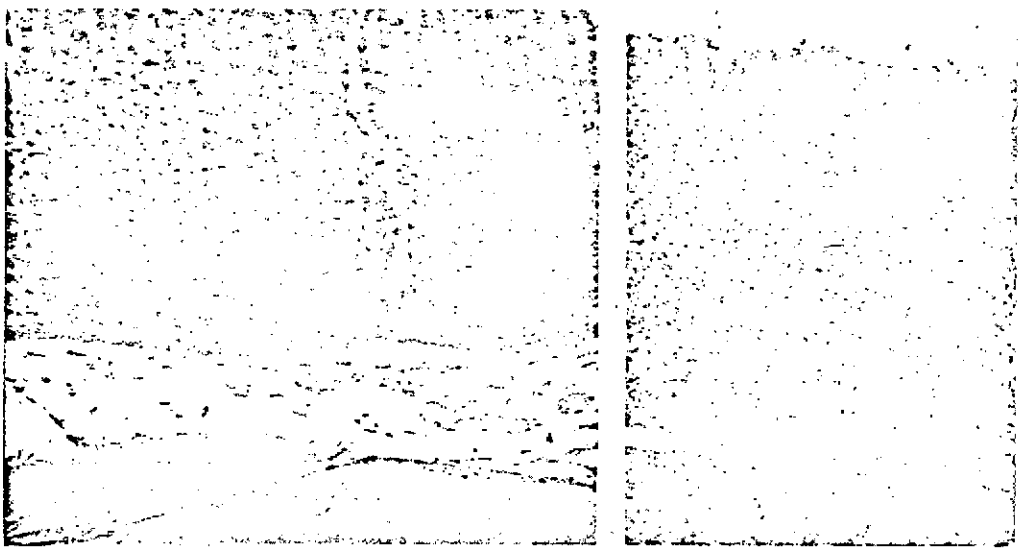

Fig. 4. Capillary pits. (a) Recent acute; (b) thrombotic, 48 hours after termination of immobilization period.

(b) Macroscopic study of lesions during healing. The le- /891
sions were reduced in depth and extent, to the point of total disappearance, in direct proportion to the length of time the animals were allowed to live before sacrifice.

Stage 1. Recent ulcer, as previously described. This was virtually never observed beyond 48 hours after release.

Stage 2. Beginning of healing process: the central coagulum was in the process of dispersion. The edges of the ulceration were regular, continuous, and not "shredded." This was the appearance at 24 and at 48 hours.

Stage 3. Active cicatrization: there were granulations along the edges of the ulcer, which were extremely congestive. The epithelium had only partially grown back in the fundus, which was clear of hematic debris. This was the predominant appearance on the second to fifth days.

Stage 4. Healed ulcer: the shape of the lesions remained visible and the depressions were more accentuated in appearance because of the granulation of the edges. On the other hand, the congestion had disappeared and the fundus was completely clean, with the epithelium grown back, so that the overall depression was identical in color to the rest of the mucosa.

This was the usual appearance on the third to the ninth days.

Finally, in some cases the stomach appeared completely undamaged. However, a whitish trace with accentuation of the vascularization sometimes marked the final scar.

(c) Chronologic development of lesions. First it should be noted that in no case was chronic ulceration observed. Under normal conditions, if the animal survived (which was virtually always the case), the cure was permanent. Of course, there was no attempt to trace the development of the lesions in a single animal by successive endoscopy. But it should be remembered that the 24-hour immobilization period resulted in nearly a 90% incidence

of ulceration, and that consequently the appearance of the lesions observed in animals sacrificed at intervals after the end of immobilization should be considered further developmental stages of the initial ulceration process.

The overall results dealing with this problem are given in Table I and Fig. 6.

These results pertain to 146 rats distributed in seven series of 20 to 25 animals.

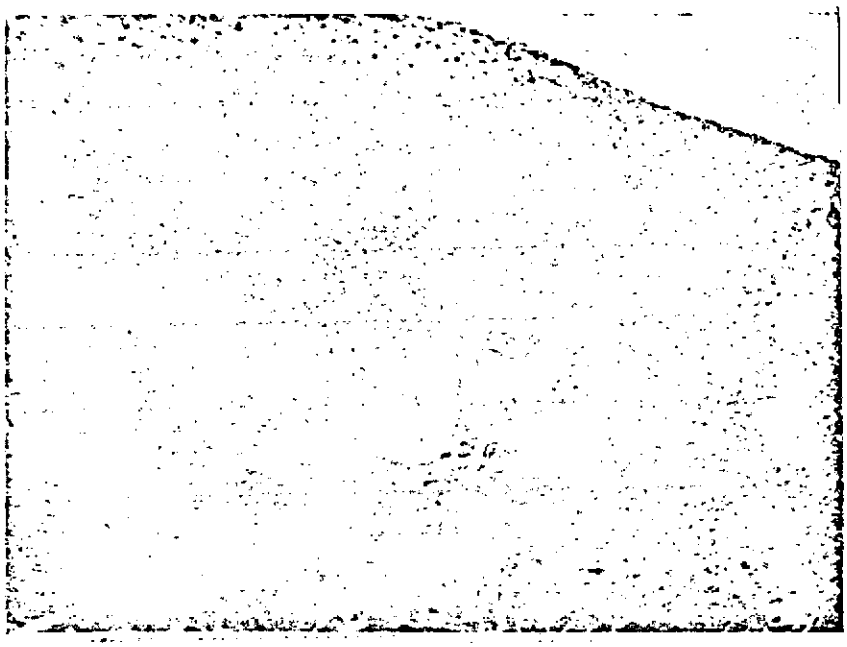

Fig. 5. Fatty infiltration and hepatic vacuoles.

The most striking phenomenon, as might be expected, was an increase in the percentage of animals showing either no damage or healed lesions as a function of time. The ratio of 5 in 20 for the first 24 hours increased to 12 in 20 for the fifth day, 14 in 20 for the sixth and 20 in 25 for the ninth.

Inversely, the number of progressive ulcers in Stages 1 and 2 decreased as a function of the length of time since the

TABLE I

Development of Gastric Lesions After the End of Immobilization

1 Jours après levée contrainte.	2 Nombre animaux.	3 Stade I.	Stade II.	Stade III.	Stade IV.	4 E. indemne.
1	20	8	5	2	4	1
2	20	3	2	5	5	5
3	20	1	1	10	4	4
4	21	2	1	11	3	4
5	20			8	6	6
6	20			6	4	10
9	25			5	8	12

Key: 1. Days after end of immobilization.
2. Number of animals.
3. Stage I (typ.).
4. Undamaged.

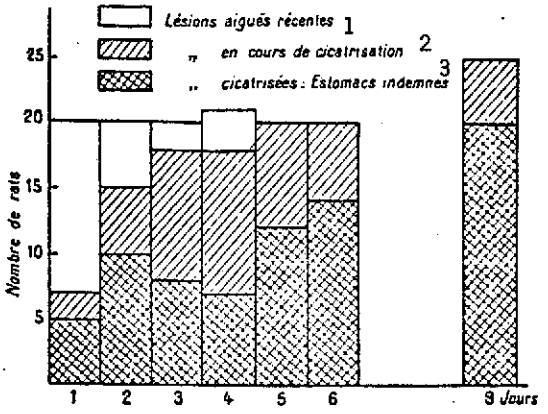

Fig. 6. Distribution of various types of lesions after release from immobilization.

Key: 1. Recent acute lesions.
2. Lesions in course of healing.
3. Healed lesions. Undamaged organs.
4. Number of rats.
5. Days.

end of the immobilization period, a fact of some interest for further experimental study. The ratio of 13 in 20 after the first 24 hours became 5 in 20, then 2 in 21 and 3 in 19, and finally was no longer observed after the fourth day.

It should be recognized that it was sometimes difficult to distinguish between the various stages and that this difficulty was increased by the fact that some animals had multiple lesions healing at different rates from one area of the organ to another. These cases were few, however, and they did offer some advantage in that they permitted an interpretation of certain atypical macroscopic appearances.

B. Microscopy of the Gastric Lesions.

Consideration will first be given to a general description of the ulcer and the neighboring mucosa.

1. General description. The ulceration consisted of a superficial slough which virtually never extended beyond the muscular mucosa. The nectrotic area was sometimes infiltrated by a small quantity of red blood cells. Its complete separation was relatively seldom noted prior to 24 hours after immobilization, and it then left a distinct parietal defect.

The peri-ulcerous reactions were extremely short-lived and usually not very severe: localized edema of the submucosa, discrete perivascular infiltrates.

The capillaries were modifed considerably throughout the mucosa, both adjacent to and at a distance from the ulcer: (a) "capillary pits," that is, severe vasodilatation of the vertical intramucous capillaries providing direct communication from the vessels of the submucosa to the gastric lumen subsequent to rupture of the cell layer closest to the surface. These vascular courses were physiologically present but not visible in this cell layer, filled with red blood cells and productive of the macroscopic hemorrhages whose occurrence we have noted in the absence of ulceration. (b) Less frequently, diffuse vasodilatation of the horizontal capillaries of the necks of the glands or the submucosic vessels. (c) Finally, rarely, intramucous interstitial hemorrhage without slough.

2. Variations in lesions found in the present experiments. (a) Seven-hour control animals (30). Half of the lesions observed consisted of simple sloughs with an inflamed appearance which was more often visible at a distance from than adjacent to the ulcer. In 2/3 of the cases the capillary pits were well distinguished. (b) Twenty-four-hour control animals (20). The lesions were multiple. Sloughs were present in all the animals. Definite ulcerations, however, were associated with the sloughs in only half the cases. The inflammation was not particularly marked. In

Fig. 7. Presence of vacuoles in renal tubular epithelial cells.

particular, capillary pits were visible only in 2/5 of the cases. (c) Vagotomy and suprarenalectomy did not modify the histologic appearance of the lesions. (d) However, this was not the case with nephrectomy (19 cases). Notable in addition to the lower in- /893
cidence of ulceration were the small depth of the ulcers, the similarity in appearance of the scars, and the absence of any apparent inflammation or any capillary pits. (e) Lesions in incipient stages. Some animals were sacrificed two and three hours after immobilization was begun, and some of these already showed ulceration. The most striking histologic pattern consisted in the size and number of the capillary pits.

In conclusion, although the ulcers induced by forced immobilization primarily occurred in the form of a predominant nectrotic lesion which was subsequently separated, one is struck by the extent and early appearance of functional capillary disturbances.

3. Development of lesions after termination of immobilization period (50 animals). The ulcers healed by a double process of granulation in the fundus of the crater and sloughing of the epithelial layer. In short, the filling tissue underwent glandular redifferentiation.

Inflammation became progressively less frequent in the area surrounding the lesion. The capillary pits, on the other hand, did not disappear, but showed a process of intracavitary "coagulation" with lysis of red blood cells resulting in the formation of relatively extensive vertical fibrous partitions.

From the sixth day on, only thrombotic capillaries and mucosic areas in the course of redifferentiation remained.

C. Microscopy of Other Visceral Lesions (Liver, Kidney)

The number of ulcers induced experimentally by immobilization was comparable to the number of experimentally induced extragastric lesions. However, it should be noted that no macroscopic or microscopic changes in other segments of the digestive tract were observed.

1. Liver. The lesions had the same characteristics for all the series studied: (a) dilatation of the vascular sinuses with congestion; (b) fatty infiltration, usually in fine particles but sometimes massive, predominantly in the periportal regions; (c) periportal inflammatory infiltrates.

However, sharp variations in incidence were often noted, depending on the type of test. Fatty infiltration was observed in 80% of the seven-hour control animals and in 100% of the 24-hour controls. The rate of occurrence was very slight, on the other hand, in animals on whom suprarenalectomy was performed.

Nephrectomy and vagotomy appeared to be accompanied by very slight sinus congestion. Periportal inflammation was especially distinct in animals on whom suprarenalectomy was performed.

2. Kidney. There was definite congestion of the kidney in all the series, occurring equally in both the glomuli and the tubulae; this may have been due to the method of sacrificing the animals.

An extremely characteristic lesion consisted in the presence of fine vacuoles in the tubular epithelial cells which was related to the presence of fats only in some cases (Fig. 7). An identical phenomenon was observed in rats on whom pyloric ligation was performed [5], where it was considered to be a possible indication of anoxia in the kidney.

This phenomenon was found in only 60% of the cases in the course of development of the ulceration, after immobilization periods of both 7 and 24 hours.

Discussion

The ulcer induced by forced immobilization thus appears to be a lesion of the glandular area of the stomach in the rat, to the exclusion of any other gastric lesion or lesion of the digestive tract.

The ulcerations are usually multiple with relatively fixed sites, similar in both appearance and topography to lesions induced by phenylbutazone. They never become perforated, and histologically they never extend beyond the muscular mucosa. In its initial stage the lesion appears to take the form of a slough whose detachment results in a parietal defect. The incidence of inflammation in the neighborhood of the lesion is minimal. On

the other hand, capillary disturbances which are often quite severe precede the appearance of the ulcerations, coexist with them at seven hours of forced immobilization, and decrease in frequency at 24 hours.

It should be recognized that in general this study offers only minimal information on the pathogenesis of gastric lesions.

Capillary alterations of the "capillary pit" type have been noted in a number of techniques for inducing ulcers in the glandular region [3, 4]. These are definite indications of severe functional disturbances, but this fact is not sufficient to confirm that the slough which permits growth of the ulcer is due solely to these circulatory modifications. The superficial location of the lesions permits rapid contact with chlorhydropeptic secretions, which increases the change. However, the absence of cell reactions in the vicinity of the ulcer after 24 hours is very surprising and may well denote a partial inhibition of local defense mechanisms during immobilization. This would explain the exacerbation of the lesions as a function of time while the capillary disturbances actually appear to diminish.

Some of our results, on the other hand, permit a precise evaluation of ulcers induced by forced immobilization as a pharmacodynamic test. (1) In evaluating a "positive" result, it is our opinion that little weight should be given to the number and extent of the ulcerations. A single punctiform ulcer is as significant as are multiple ulcerations distributed over both surfaces. Prolongation of the immobilization period is the determining factor in this expansion. For series with comparable immobi- /894
lization periods the number of lesions will thus primarily be a function of the susceptibility of the individual animal, which determines the time at which the lesions will appear. Thus only negative results, that is, complete absence of any ulcerous lesion,

are of importance. The influence of any individual factors is therefore excluded. (2) Study of the healing process, if performed on a sufficient number of animals, will furnish completely reproducible results, since a 100% survival rate is assured after release from immobilization. Macroscopic and microscopic study will thus permit evaluation of the healing or inhibitory capabilities of drugs or nutritional factors.

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